Progetto ricerca di base - BLOCKCHAIN4FOODCHAIN

Laboratory of Food Chemistry

13.10.2015 IT Gazzetta ufficiale dell'Unione europea L 266/9

REGOLAMENTO DELEGATO (UE) 2015/1830 DELLA COMMISSIONE

dell'8 luglio 2015

che modifica il regolamento (CEE) n. 2568/91 relativo alle caratteristiche degli oli d'oliva e degli oli di sansa d'oliva nonché ai metodi ad essi attinenti

Legal limits for oils deriving from processing of *Olea europea* fruits

ALLEGATO

«ALLEGATO I

CARATTERISTICHE DEGLI OLI DI OLIVA

Categoria	Etil esteri degli acidi grassi (EEAG) (*)	Acidità (%) (*)	Numero dei perossidi mEq O_2/kg (*)	Cere mg/kg (**)	2 gliceril monopalmitato (%)	Stigmastadieni mg/kg (1)	Differenza: ECN42 (HPLC) e ECN42 (calcolo teorico)	K_{232} (*)	K_{268} o K_{270} (*)	Delta-K (*)	Valutazione organolettica Mediana del difetto (Md) (*)	Valutazione organolettica Mediana del fruttato (Mf) (*)
1. Olio extra vergine di oliva	EEAG ≤ 40 mg/kg (campagna 2013-2014) (2) EEAG ≤ 35 mg/kg (campagna 2014-2016) EEAG ≤ 30 mg/kg (campagne successive al 2016)	≤ 0,8	≤ 20	C42 + C44 + C46 ≤ 150	≤ 0,9 se % acido palmitico totale ≤ 14 % ≤ 1,0 se % acido palmitico totale > 14 %	≤ 0,05	≤ |0,2|	≤ 2,50	≤ 0,22	≤ 0,01	Md = 0	Mf > 0

DETERMINATION OF ACIDITY AND PEROXIDE VALUES OF EVOO SAMPLE -REG. (UE) 2015/1830-

The **Acidity Value** (AV) determination =
official measure of the **hydrolytic phenomena** of triacylglicerols of EVOO.
(legal limit = 0.8% espressed in oleic acid for EVOO).

The **Peroxide Value** (PV) determination =
official method of the **primary oxidation** of triacylglicerols of EVOO
(legal limit = 20 meq O_2/kg oil).

EVOO sample:
Delivered on 09-02-2023 (T0)

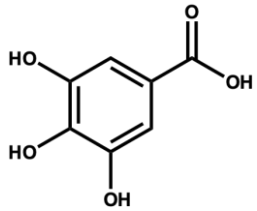
EVOO Sample – Rocca di Casalina	Peroxide value (meq O_2/Kg) mean value ± SD	Olive oil acidity (% oleic acid) mean value ± SD
T0	5.9 ± 0.12	0.48 ± 0.01

TPC (Folin-Ciocalteu spectrophotometric assay)

The total phenol content (TPC) of EVOO was determined with the **FOLIN-CIOCALTEU REAGENT**, a mixture of phosphomolibdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$).

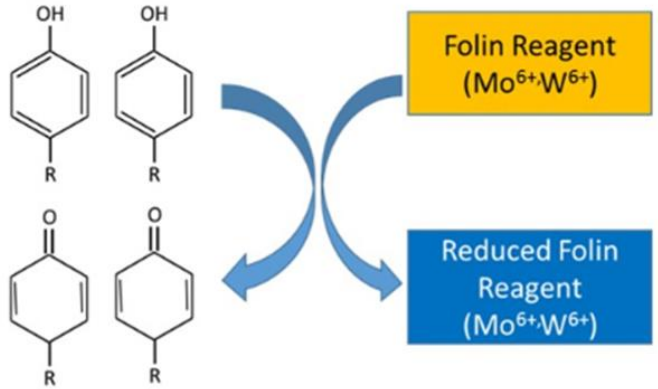
Phenols reduce the **Folin-Ciocalteu reagent** forming a **blue mixture of oxides (Mo_8O_{23} e W_8O_{23})**

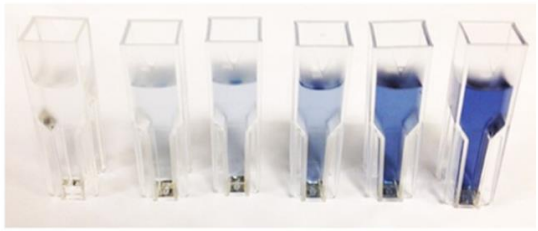
✓ The TPC values are determined from a calibration curve prepared with **GALLIC ACID standard solutions**, and the results expressed as mgGAE/kg oil.

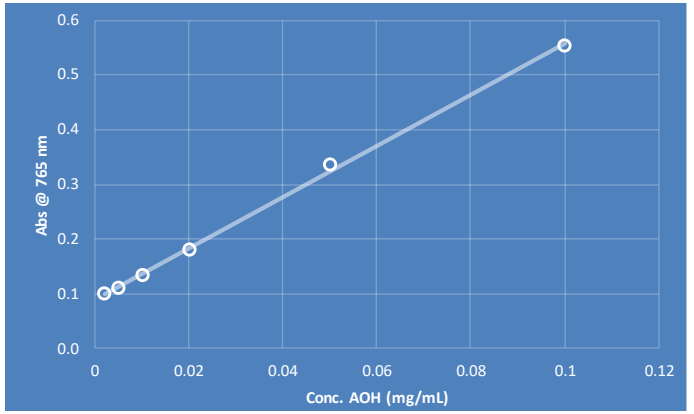


GALLIC ACID

✓ **The HIGHER the absorbance at λ=765 nm, the HIGHER the TPC of the investigated extract**







calibration curve built up by using GA solutions

Determination of Antioxidant Activity

The determinations of antioxidant properties of EVOO were carried out applying three different *in vitro* complementary spectrophotometric assays:

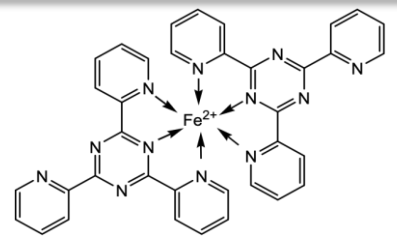
1. FRAP spectrophotometric assay

2. ABTS spectrophotometric assay

3. DPPH spectrophotometric assay

1. FRAP spectrophotometric assay (Ferric Reducing Antioxidant Power)

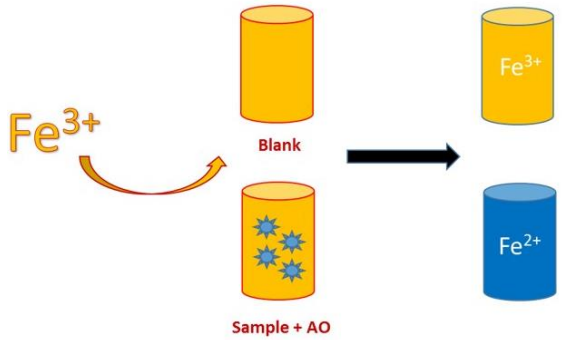
FERRIC REDUCING ANTIOXIDANT POWER (FRAP) method is based on the capacity of phenols to reduce the **Fe(III) 2,4,6-*tris*-(2-pyridyl)-*s*-triazine [Fe(III)-$(TPTZ)_2$]$^{3+}$** complex to **Fe(II) 2,4,6-*tris*-(2-pyridyl)-*s*-triazine [Fe(II)-$(TPTZ)_2$]$^{2+}$** complex.

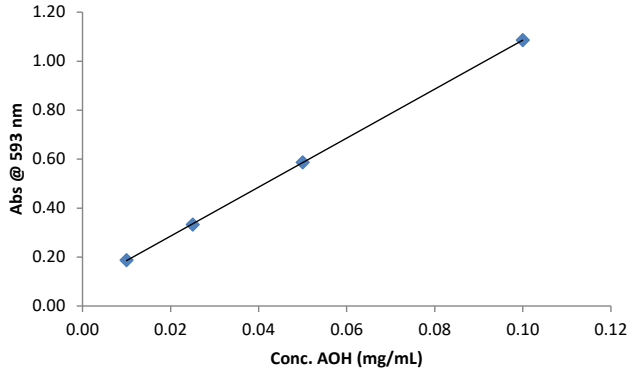


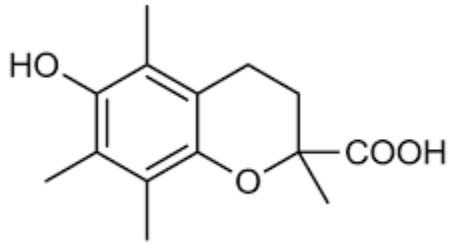
[Fe(II)-$(TPTZ)_2$]$^{2+}$
Blue

✓ The values are determined from a calibration curve prepared with **TROLOX standard solutions** (results expressed as mgTE/kg oil)

✓ **The HIGHER the absorbance at λ=593 nm, the HIGHER the total antioxidant capacity of the investigated extract (from yellow to blue)**







TROLOX

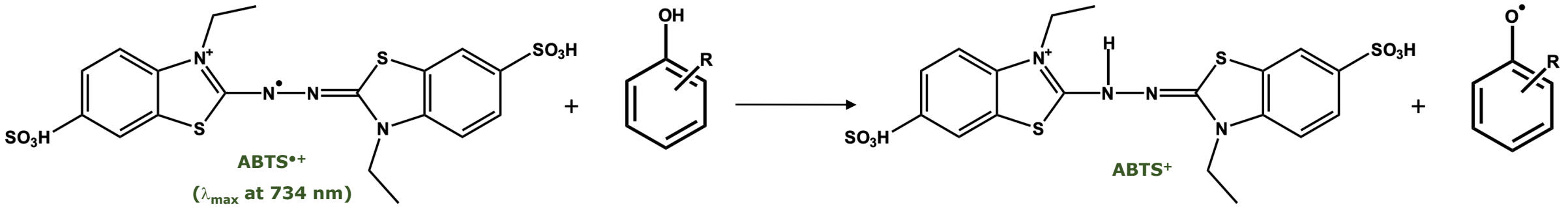
2. ABTS spectrophotometric assay

TROLOX EQUIVALENT ANTIOXIDANT CAPACITY (TEAC/ABTS) assay is based on the ability of phenols to act as ABTS free radical-scavenger.

The ABTS radical ($ABTS^{\bullet+}$) is generated in aqueous phase by reacting a strong oxidizing agent (e.g., potassium persulfate, $K_2S_2O_8$) with the **$ABTS^{+}$ (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)**.

Total antioxidant capacity values are determined from a calibration curve prepared with TROLOX standard solutions, and the results expressed as mgTE/kg oil.

Radical Scavenging Mechanism



The LOWER the absorbance at λ=734 nm,

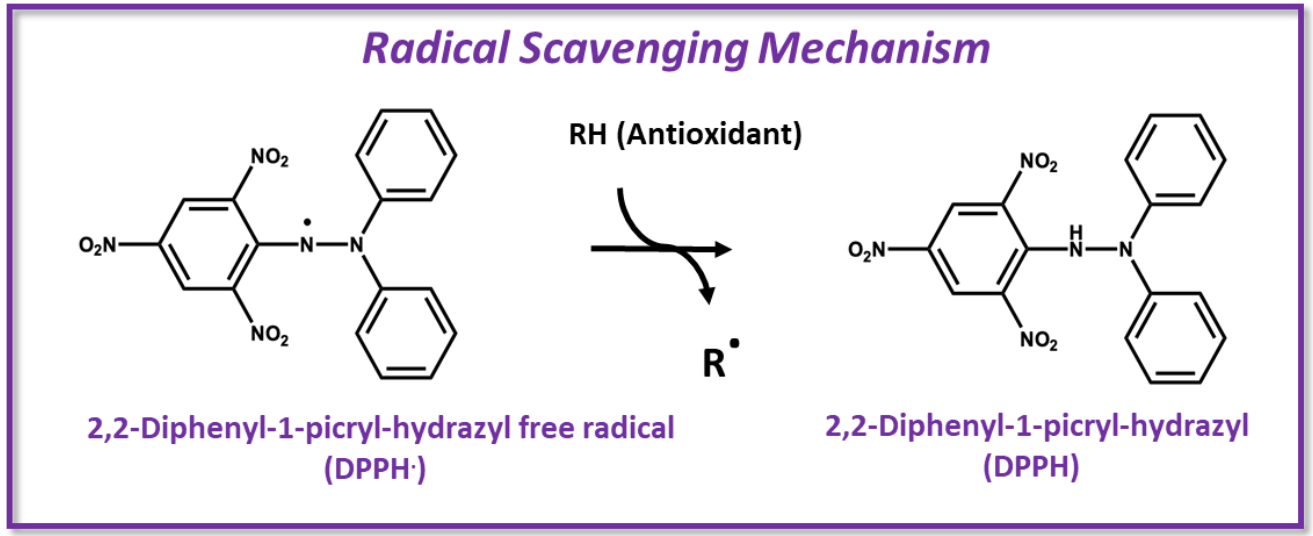
the HIGHER the total antioxidant capacity of the investigated extract

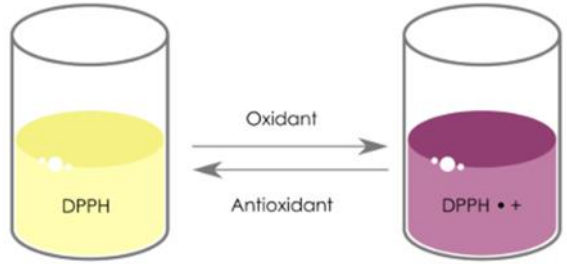
3. DPPH spectrophotometric assay

2,2-DIPHENYL-1-PICRYL-HYDRAZYL (DPPH) free radical assay is based on the ability of phenols to act as free radical scavengers or hydrogen donors.

The odd electron in the DPPH· free radical produces a strong absorption maximum at 517 nm (**purple colour**).

The colour turns **from purple to yellow** as the molar absorptivity of the DPPH· radical at 517 nm reduces as a result of the pairing of the odd electron of DPPH· radical with a H atom from a free radical scavenging antioxidant to produce DPPH-H. Total antioxidant capacity values are determined from a calibration curve prepared with TROLOX standard solutions, and the results expressed as mgTE/kg oil.





The LOWER the absorbance at 517 nm, the HIGHER the total antioxidant capacity of the investigated extract

Determination of TPC and antioxidant activity of EVOO sample

EVOO sample:
Delivered on 09-02-2023 (T0)

Then stored in:

EVOO Sample – Rocca di Casalina	TPC (mg GAE/kg) mean value ± SD	DPPH (mg TE/kg) mean value ± SD	ABTS (mg TE/kg) mean value ± SD	FRAP (mg TE/kg) mean value ± SD
T0	179.67 ± 7.07	171.10 ± 10.38	302.67 ± 11.80	218.26 ± 1.58

Rocca di Casalina EVOO: mix of *cultivars* (Leccino, Moraiolo, Frantoio)

Comparison with TPC of other EVOO samples

TPC mg GAE/kg	*Cultivars*	References
180	**Moraiolo, Apulia (Italy)**	**Baiano et al., 2009**
78-199	**Moraiolo**	**Hanbook Olive oil - Springer**
135	Frantoio, Umbria (Italy)	Ninfali et al., 2001
97.63–236.41 (Sicily) 268.63–509.00 (Apulia) 171.16–573.20 (Tuscany) 161.82–298.23 (Lazio)	Thirty mono- and multivarietal and PDO extra-virgin olive oils samples were collected from Italian producers located in different areas belonging to the harvest year 2017.	Fanali et al., 2018
138-278	8 EVOOs (monocultivar) from Apulia	Negro et al., 2019
112-163	Carolea (Calabria, Italy)	Dini et al., 2020
70-96	Leccino (Calabria, Italy)	Dini et al., 2020
133-421	4 cultivar EVOO (Sardinia, Italy)	Tuberoso et al., 2016
158-395	2 Tunisian cultivars	Nakbi et al., 2010
112-163	Carolea (Calabria)	Dini et al., 2020
70-96	Leccino (Calabria)	Dini et al., 2020
133-421	4 cultivar EVOO (Sardegna)	Tuberoso et al., 2016
180 (T zero) **144 (6 months)** **38 (12 months)**	**Moraiolo, Cerignola (Puglia) (STORAGE)**	**Baiano et al., 2009**

Rocca di Casalina EVOO: mix of *cultivars* (Leccino, Moraiolo, Frantoio)

Comparison with antioxidant activity of other EVOO samples

FRAP assay mmolTE/kg	Cultivars	References
0.36-2.95	4 cultivar EVOO (Sardegna)	Tuberoso et al., 2016
0.70-2.22	Arbequina EVOO from Spain	Borges et al., 2017
0.38–1.67	Spanish monovarietal EVOO	Ramírez-Anaya et al., 2019

ABTS assay mmolTE/kg	Cultivars	References
0.46-0.76	Carolea from Calabria (Italy)	Dini et al., 2020
0.24-0.36	Leccino from Calabria (Italy)	Dini et al., 2020
0.09-1.88	Four cultivars from Sardinia (Italy)	Tuberoso et al., 2016
0.20-0.73	Arbequina from Spain	Borges et al., 2017
1.17	EVOO (Spain)	Ramírez-Anaya et al., 2019
0.61-2.42	Two Tunisian cultivars	Nakbi et al., 2010

DPPH assay mmolTE/kg	Cultivars	References
0.342-0.556	Carolea from Calabria (Italy)	Dini et al., 2020
0.266-0.388	Leccino from Calabria (Italy)	Dini et al., 2020
0.25-1.17	Four cultivars from Sardinia (Italy)	Tuberoso et al., 2016
0.52-1.58	Arbequina from Spain	Borges et al., 2017

WORK IN PROGRESS …

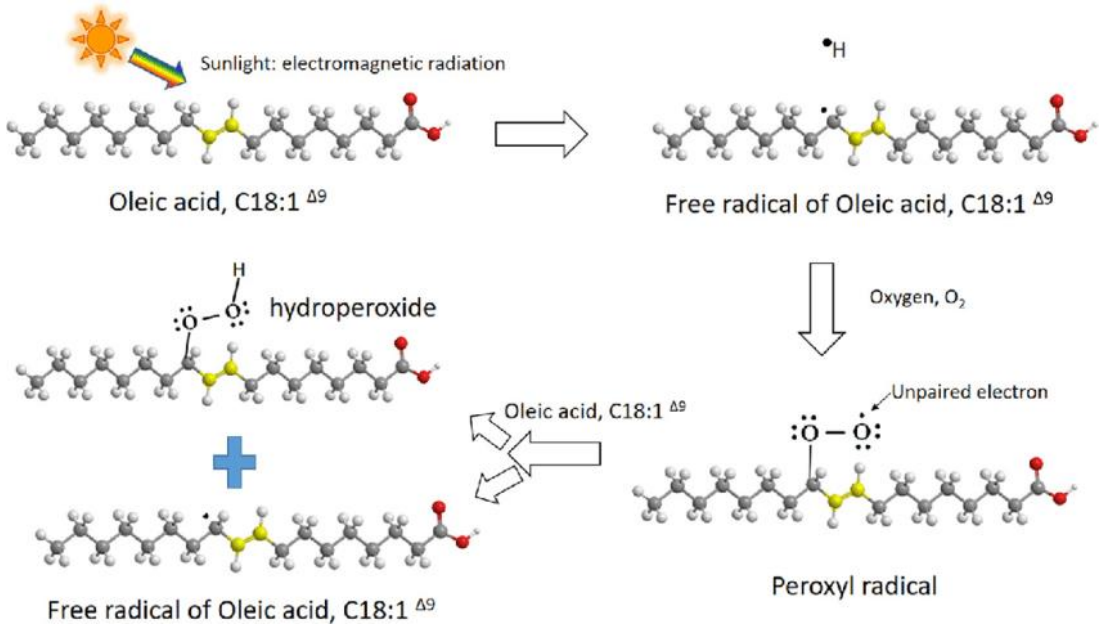
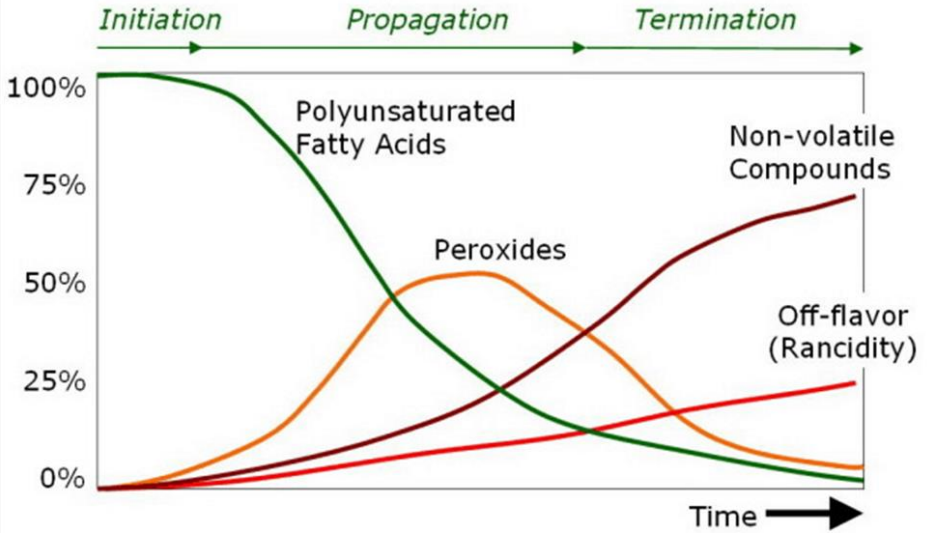


Photo-oxidation and autoxidation oleic acid and formation of peroxide

Autoxidation: initiation, propagation, termination



NEXT STEPS …

1

Development and application of a RP-HPLC-PDA method to characterize the phenol pool of EVOO sample, followed by confirmation *via* HRMS analysis:

- ❖ Optimization of experimental conditions (column selection and gradient program) on a mixture of standard phenolic compounds commonly present in EVOO
- ❖ Evaluation of the phenolic profile of EVOO sample at the starting time (T0)
- ❖ Comparison of chromatographic profile with photooxidised EVOO samples (T1, T2, T3)
- ❖ Quantitative analysis of the hydroxytyrosol as the main quality marker of EVOO
- ❖ Quantitative determination of other identified phenolic compounds through the use of proper calibration curves
- ❖ Correlation studies between the results of spectrophotometric assays and the HPLC-based determinations

2

Evaluation of TPC and antioxidant properties in plasma samples obtained from patients treated with Rocca di Casalina EVOO (provided by Prof. Vaudo and Dr. Cavallo)